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# A new species of *Pluteus* (*Pluteaceae, Agaricales*) from Mexico

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**Abstract**— A new species, *Pluteus nevadensis* from subtropical and pine forests in Mexico, is described and compared with similar taxa. Phylogenetic analyses of the ITS rDNA sequence data support the classification of this new taxon in *Pluteus* section *Celluloderma*.

**Key words**— cystidia, *Pluteus aurantiorugosus*, *Pluteus horakianus*

## Introduction

*Pluteus* Fr. is an agaric genus typically classified in the *Pluteaceae* Kotl. & Pouzar (Singer 1986). It is characterized by the free and pink-coloured lamellae, absent volva and annulus, and the convergent lamellar trama. It has a worldwide distribution that includes saprobic species, the majority of them lignicolous.

Until now, 33 species of the genus *Pluteus* have been reported from Mexico. Some of the species that are only known from this country include *Pluteus horridus* Singer, *P. leucocyanescens* Singer, *P. multistriatus* Murrill, *P. nitens* Pat., *P. triplocystis* Singer, and more recently *P. neotropicalis* Rodr.-Alcánt. and *P. horakianus* (Patouillard 1898, Murrill 1911, Singer 1973, Rodríguez et al. 2008, 2009).

Continuing with our study of *Pluteus* in Mexico, a careful study of some Mexican collections previously reported as *P. aurantiorugosus* revealed that some specimens represent a new species. Using morphological and molecular characters, this new species, *Pluteus nevadensis*, is described and reported from subtropical and pine forests in the states of Guerrero and Jalisco. Based on the infrageneric classification used by Singer (1986), which is corroborated with phylogenetic analyses of the internal transcribed spacer (ITS) of rDNA sequence data (Rodríguez et al. 2009), *P. nevadensis* is included in *Pluteus* section *Celluloderma* Fayod.

## Material and methods

### Morphology

Micromorphological observations were made from sections of the basidiomata mounted in 3% KOH. The terms for the descriptions are mainly those of Vellinga (1998) and in some cases those of Largent et al. (1977). Basidiospores shape was determined according to the Q (length-width ratio) (Bas 1969) of at least 20 mature and randomly selected basidiospores. The length of basidia measurements includes sterigmata. Illustrations were made with the aid of a drawing tube. The herbaria and author abbreviations follow Holmgren et al. (1990) and Kirk & Ansell (1992), respectively.

### DNA extraction

Total genomic DNA was extracted from herbarium specimens following the protocol described in Aljanabi & Martinez (1997) with some modifications (Torres-Torres et al. 2009). Pellet DNA was resuspended in 30–80 µl of TE. The raw DNA was then diluted 1:2 in MilliQ water to reduce pigment concentration.

### PCR amplification

The internal transcribed spacer (ITS), containing the ITS1, 5.8S and ITS2 regions of rDNA, was amplified by the polymerase chain reaction (PCR), using the pair primers ITS1F-ITS4 to amplify the entire ITS (Vilgalys & Hester 1990) or ITS5-ITS5.8S to amplify the ITS1 and ITS5.8SR-ITS4S to amplify the ITS2 (Gardes & Bruns 1993, Kretzer et al. 1996). The PCR reaction volumes were adjusted to 25 µL, consisting of 16.9 µL of MilliQ water, 2.4 µL of 10X reaction buffer (100 µM Tris, 500 µM KCl), 1.2 µL $MgCl_2$ (Applied Biosystems), 1.2 µL of 5 mM dNTPs, 0.1 µL of Taq DNA polymerase 5U/µL (Applied Biosystems), 0.5 µL of each 10 µM primer, 1.2 µL of BSA (bovine serum albumine) (New England Bio Labs), and 1 µL of DNA template.

PCR amplifications were performed in a MJ Research PTC 200 thermocycler as described by Rodríguez et al. (2009). Amplification from ITS region was confirmed under UV light using 1.5% agarose (NuSieve, FMC Bioproducts) gel electrophoresis in the presence of ethidium bromide. PCR products were purified with GFX$^{tm}$ purification kit (Amersham Biosciences) according to the instructions provided.

### Sequencing

Sequencing reactions were performed with BigDyeTM Terminator v3.1 Cycle Sequencing (Applied Biosystems) following the manufacturer's protocols with the same primers as those used in the PCR. Sequencing reactions were purified with AutoSeqTM G-50 column (Amersham Biosciences) with 18 µL of formamide being added. Sequences were obtained by capillary electrophoresis on an ABI-Prism 310 Genetic Analyzer (Applied Biosystems). Three new sequences were generated, thirteen were from Rodríguez et al. (2009) and four were retrieved from GenBank. New sequences were deposited in GenBank with accessions numbers GU551941-GU551943 (Table 1). Resulting chromatograms were edited using Chromas 1.45 (McCarthy 1996–1998) and manually corrected when necessary. The assembly of the sequence fragments and the alignment of all sequences were carried out using MacClade 4.0 (Maddison & Maddison 2000).

## Molecular analyses

One dataset was prepared based on 20 ITS rDNA sequences of 17 taxa: 15 sequences from 12 *Pluteus* species, one *Volvariella*, one *Leucoagaricus* and three *Entoloma* taxa (Table 1). Phylogenetic trees were inferred with PAUP* 4.0b10 (Altivec) (Swofford

Table 1. Species used in the phylogenetic analysis.

| DNA code | Species | Origin | Collector, number (herbarium), collection date | GenBank accession |
|---|---|---|---|---|
| **113** | *P. albostipitatus* var. *poliobasis* Singer | Mexico | O. Rodríguez 1545 (IBUG), 2006 | FJ375244[a] |
| **160** | *P. aurantiorugosus* (Trog) Sacc. | Spain | J.C. Zamora s.n (AH), 2001 | FJ375248[a] |
| **6** | *P. cervinus* (Schaeff.) P. Kumm. | Mexico | L. Guzmán-Dávalos 3513 (IBUG), 1986 | FJ375241[a] |
| **242** | *P. diverticulatus* Corriol | France | 0092579 (holotype, PC), 1950 | FJ375247[a] |
| **58** | *P. horakianus* Rodr.-Alcánt. | Mexico | L. Guzmán-Dávalos 7488 (IBUG), 1998 | FJ375250[a] |
| **60** | *P. horakianus* | Mexico | L. Guzmán-Dávalos 7271 (holotype, IBUG), 1998 | FJ375251[a] |
| **65** | *P. nevadensis* Rodr.-Alcánt. | Mexico | V. Calderón s.n. (FCME-13128), 1984 | GU551941 |
| **67** | *P. nevadensis* | Mexico | O. Vargas 525 (holotype, IBUG), 1991 | GU551942 |
| **114** | *P. nigrolineatus* Murrill | Mexico | O. Rodríguez 1548 (IBUG), 1996 | FJ375245[a] |
| **222** | *P. pellitus* (Pers.) P. Kumm. | Mexico | J. García 9934 (IBUG), 1996 | FJ375243[a] |
| **100** | *P. petasatus* (Fr.) Gillet | Mexico | O. Rodríguez 2587 (IBUG), 2004 | FJ375242[a] |
| **236** | *P. pulverulentus* Murrill | West Indies | W.E. Broadway (holotype, NY), 1905 | GU551943 |
| **119** | *P. romellii* (Britzelm.) Lapl. | Mexico | O. Rodríguez 1565 (IBUG), 1996 | FJ375246[a] |
| **85** | *P. thomsonii* (Berk. & Broome) Dennis | France | 95091602 | FJ375252[a] |
| **155** | *P. thomsonii* | Spain | F. Pardo s.n. (AH), 2001 | FJ375253[a] |
| **GB** | *Leucoagaricus sinicus* (J.Z. Ying) Zhu L. Yang | GB | | DQ182505[b] |
| **GB** | *Entoloma bloxamii* (Berk. & Broome) Sacc. | GB | | EF530938[c] |
| **GB** | *Entoloma nitidum* Quél. | GB | | AY228340[d] |
| **GB** | *Entoloma sericeum* Quél. | GB | | AF357020[e] |
| **176** | *Volvariella gloiocephala* (DC.) Boekhout & Enderle | USA | L. Guzmán-Dávalos 8444 (IBUG), 2000 | FJ375254[a] |

[a] Rodríguez et al. (2009): [b] From Matheny & Hibbett in 2005; [c] From Denis et al. in 2007; [d] From Acorn et al. in 2003; [e] From Hofstetter et al. in 2002.

2000) and were rooted with species of *Volvariella*, *Leucoagaricus* and *Entoloma*. Branch-and-bound searches were performed using the criterion of maximum parsimony with furthest addition sequence, branches collapsed if maximum branch length is zero, only minimal trees were kept, and MulTrees option in effect. Gaps were treated as missing characters. Starting trees were obtained via stepwise addition. Relative branch support was estimated with 1000 bootstrap replications (Felsenstein 1985) with the same parameters previously mentioned. The initial dataset included 785 characters. For the parsimony analysis, 443 sites at both ends of the sequences and ambiguous regions were excluded. The parsimony tree scores, including tree length and consistency, retention, rescaled consistency and homoplasy indices (CI, RI, RC and HI) excluding uninformative characters, were calculated. Additionally, the percentage of sites (base pairs) differing between sequences of *P. nevadensis* and both *P. aurantiorugosus* and *P. horakianus* sequences was obtained.

## Results

### Description of the species

***Pluteus nevadensis*** Rodr.-Alcánt., **sp. nov.** Figs. 1–8

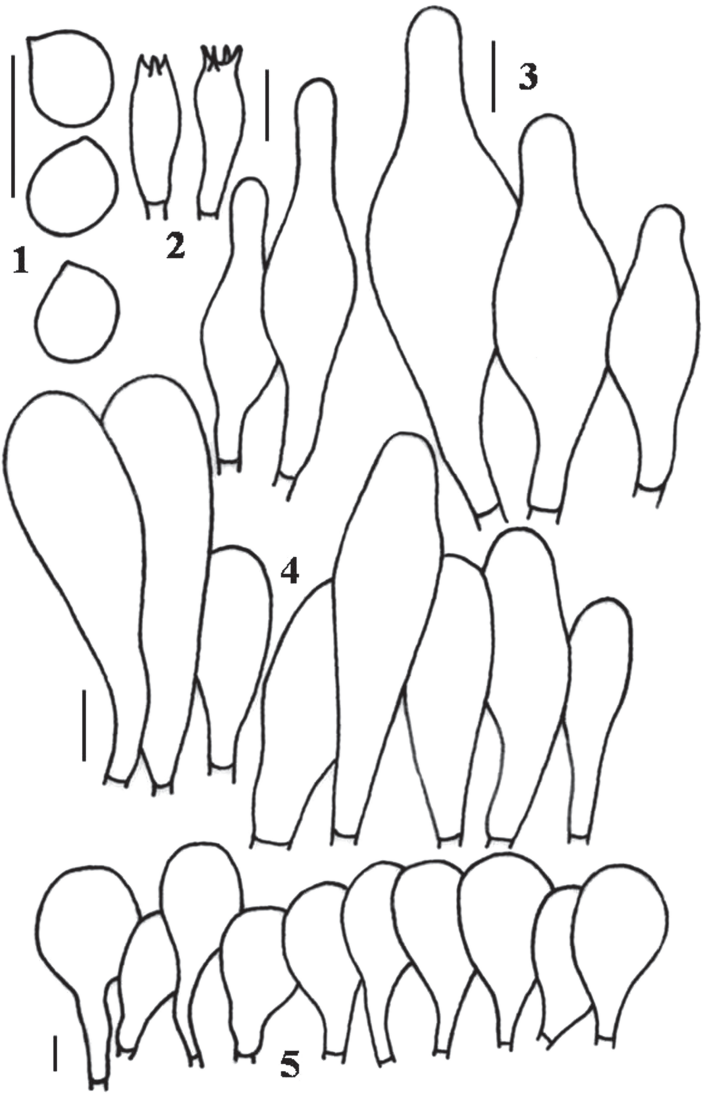
Mycobank MB515446

*Pileus 15–38 mm latus, primo conic vel campanulatus, dein plano-convexus, umbonatus, rugulosus vel levis ad discum, deflexus, erosus vel planus marginem rubroaurantiacus vel brunneoruber, siccus vel humidus. Lamellae liberae, latus vel ventricosae, primo albus vel albidus, dein salmoneus-roseus, ad aciem floccosus vel fimbriatus, albidue. Stipes 6–45 × 2–6 mm, cylindricus, aequalis, curvatus, flavus vel flavobrunneus ad apex aurantiacus obscure vel aurantiacus tinctus basalis, albidus, cottoneus-strigae mycelium, levis vel fibrillosus, sericeus, siccus. Caro 1mm, albidula ad pileus vel flavobrunneus vel aureus. Odor saporque nulli. Basidiosporae 5–6.5(–8) × 4.5–6.5 µm, late ellipsoideis vel ellipsoideis, rarus globosus. Basidia 22–29 × 6.5–7.5 µm, clavata, 4-sporigera, defibulata. Cheilocystidia (24–)32–52(–61) × (8–)10–18.5(–24) µm, clavata vel angustus clavata, utriformis, subcylindricus, obovatus, hyalina. Pleurocystidia 42–75(–82) × 12–24(–27.5) µm, polymorphica, lageniformia, brevicollis vel elongatus, subfusiformia vel subutriformis, hyalina. Caulocystidia nulla. Pileipellis e epithelium cellulis clavatis vel sphaeropedunculate formantibus, 26–60 × 14–31 µm, hyalina. Fibulae nullae. Habitatio ad lignum putridum in silvis mixtis (*Pinus, Quercus*).*

**Holotypus**: Mexico, Jalisco: Municipality of Zapotlán el Grande, Nevado de Colima, El Floripondio 2100 m, 10.VIII.1991, O. Vargas 525 (IBUG).

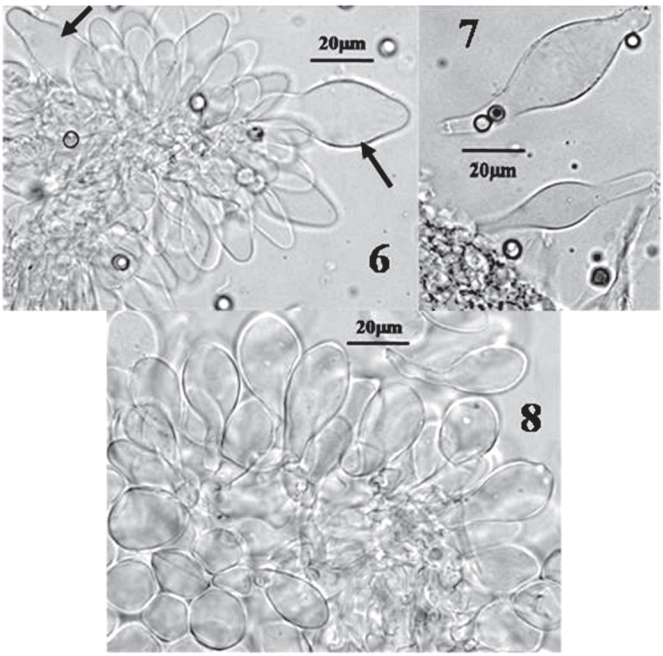
Etymology – *nevadensis*. Named after the mountain where the type material was collected.

Pileus 15–38 mm broad, conic when young, campanulate to convex or plane-convex when mature, umbonate; margin decurved, even or slightly eroded; surface dry to moist, rugulose towards the margin, with the disk rugose to smooth; sometimes white-yellowish context underneath the cuticle is visible; red-orange to reddish-orange. Lamellae free, crowed, broad to ventricose, white or whitish when young to salmon-pinkish in age; edge floccose or




Figs. 1–5: *Pluteus nevadensis* (Holotype),
1: basidiospores (× 2000), 2: basidia (× 1000), 3: pleurocystidia (× 1000),
4: cheilocystidia (× 1000), 5: pileipellis (× 500).

fimbriate, whitish. Stipe 6–45 × 2–6 mm, central, equal, glabrous to slightly fibrillose, silky, hollow, yellow or yellowish at the apex, deep orange or with orange tinges towards the base; with cottony-strigose, whitish mycelium at the




Figs. 6–8: *Pluteus nevadensis*,
6: cheilocystidia with two pleurocystidia, 7: pleurocystidia,
8: elements of pileipellis.

base. Pileus context 1 mm thick or more at the disk, fleshy, whitish. Stipe context yellowish or yellow gold. Smell and taste not distinctive.

Basidiospores 5.5–7(–8) × 4.5–6.5 µm, Q = (1–)1.09–1.2 ($L^m$ = 6.1 µm, $W^m$ = 5.2 µm), subglobose to broadly ellipsoid, rarely globose, smooth, wall thin to slightly thickened, subhyaline. Basidia 22–29(–36) × 6.5–7.5 µm (including sterigmata), clavate, 4-spored, with refringent content, hyaline. Pleurocystidia (38.5–)41.8–75(–81.8) × 11.8–24(–27.5) µm, frequent, scattered, lageniform with short or elongated neck, some subfusiform or subutriform, thin-walled, hyaline. Cheilocystidia (24–)32–55(–61) × (8–)10–18.5(–24) µm, crowded, clavate to narrowly clavate, some utriform, subcylindrical or obovoid, thin-walled, hyaline. Lamellar trama convergent Pileipellis an epithelium with elements 25.6–60 × 13.6–31.2 µm, clavate or sphaeropedunculate, generally with a long pedicel, wall thin or slightly thickened, hyaline. Oleiferous hyphae and clamp connections absent.

Material examined – MEXICO: GUERRERO, Municipality of Chilpancingo, Cerro Palo Hueco, Omiltemi, 14.VII.1984, *V. Calderón s.n.* (FCME-13128). JALISCO: Municipality of Zapotlán el Grande, Nevado de Colima, El Floripondio, 23.VII.1988, *L. Guzmán-Dávalos 4261* (IBUG), 10.VIII.1991, *O. Vargas 525* (holotypus, IBUG).

**Molecular analyses**

A Branch-and-Bound search of the ITS rDNA sequence data generated four most parsimonious trees with a tree length of 247 steps. Of the 342 sites considered for the analysis, 54 were parsimony informative. Excluding uninformative characters CI = 0.555, RI = 0.7478, RC = 0.490 and HI = 0.445. Figure 9 shows one of the trees, which has the same topology of the other three, except in the placement of *P. pulverulentus*. This species always was placed within section *Celluloderma*, but its position is not resolved in the strict consensus tree. The bootstrap support for the clades is from 55 to 100%, except for one clade that is below 50%. *Pluteus nevadensis* is placed in the clade representing section *Celluloderma* along with the morphologically similar species *P. aurantiorugosus*, but *P. nevadensis* has a sister relationship with *P. horakianus* in a different subclade.

The analysis of the ITS region shows that the percentage of sites (base pairs) differing between *P. nevadensis* and both *P. horakianus* and *P. aurantiorugosus* are 7.6% and 14.3%, respectively.

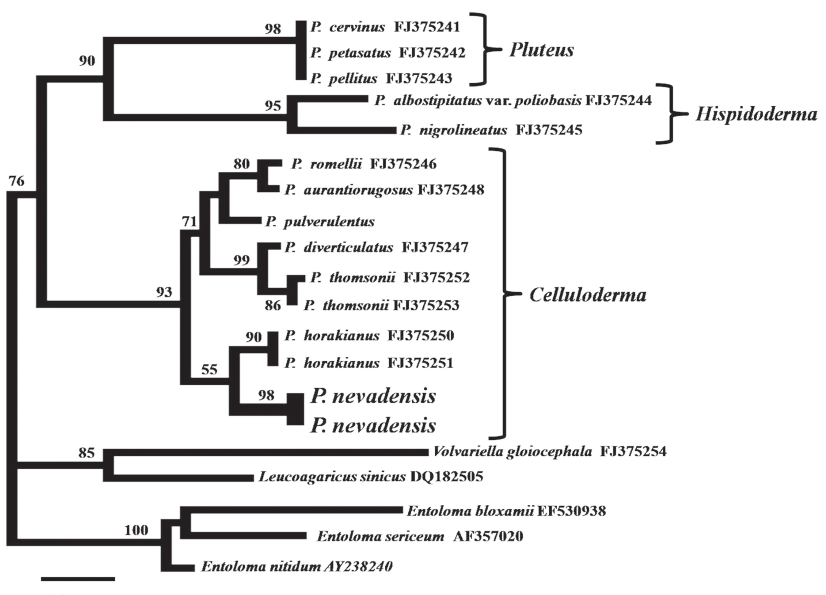



Fig. 9 One of the four phylograms resulting from a branch-and-bound search, of 12 species (15 samples) of *Pluteus*, and five outgroups, based on ITS rDNA sequence data. Tree length = 247 steps, parsimony informative characters = 54, CI excluding uninformative characters = 0.555, RI = 0.7478, RC = 0.490. Bootstrap values > 50% obtained from a branch-and-bound search with 1000 replicates are given above each branch.

## Discussion

The Mexican collections are macromorphologically similar to *Pluteus aurantiorugosus*, so much so that all of the studied specimens (Calderón s.n., Guzmán-Dávalos 4261, Vargas 525) were previously recorded as this species (Cifuentes et al. 1989, Rodríguez & Guzmán-Dávalos 2001). *Pluteus aurantiorugosus* is the most similar taxon because of the scarlet, orange to red-orange pileus, the yellow to orange stipe, and the fimbriate and whitish lamellar margin. Micromorphologically, *P. aurantiorugosus* has a percentage of basidiospores that are oblong, cystidial shapes that are variable from clavate, broadly clavate to subfusiform and pileipellis elements that are typically globose with or without pigment. *Pluteus nevadensis* is distinguished by the lack of oblong basidiospores, the lageniform pleurocystidia and narrowly clavate cheilocystidia that are larger and more slender than those observed in *P. aurantiorugosus*, and by the more typically clavate pileipellis elements without pigment.

*Pluteus nevadensis* and *P. horakianus* are also morphologically similar fungi in the fragile basidiome, red pileus, and by the form of the pleuro- and cheilocystidia. However, *P. horakianus* is distinguished by the orange-reddish lamellar edges, the red stipe, and the pigmented pileipellis elements. Other superficially similar fungi, mainly sharing basidiome coloration, are *P. aurantiopustulatus* E. Horak, *P. aurantipes* Minnis et al., *P. flammipes* E. Horak, *P. laetifrons* (Berk. & M.A. Curtis) Sacc., and *P. laetus* Singer. Micromorphological characters such as a lack of pleurocystidia, form and size of cystidia, or different type of pileipellis readily separate *P. nevadensis* from these species. Rodríguez et al. (2009) summarized these characters for species similar to *P. horakianus*.

Previously, Rodríguez et al. (2009), based upon analyses of ITS rDNA sequence data, found that *P. horakianus* represented a distinct taxon belonging to section *Celluloderma* and that it was in a different clade than *P. aurantiorugosus*. Here, a phylogenetic analysis of the ITS region data (Fig. 9) shows that *P. nevadensis* is in a sister relationship with *P. horakianus* in section *Celluloderma*. This indicates that the two species are phylogenetically very closely related and distant to other *Pluteus* species, in particular to *P. aurantiorugosus*.

Furthermore, the analysis of the ITS region shows the percentage of ITS region sites differing between *P. nevadensis* and both *P. horakianus* and *P. aurantiorugosus* to be rather large (7.6% and 14.3%, respectively). Those percentages are high values compared with 2.2 – 4.0% between *Cortinarius* species (Vila et al. 2008).

Finally, we concluded that the examined collections of the Mexican *P. nevadensis* have enough morphological and molecular differences to be considered as a distinct new species.

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